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## *Incipient genetic isolation of a temperate migratory coastal Sciaenid fish (Argyrosomus inodorus) within the Benguela Cold Current system*

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**Title:** Incipient genetic isolation of a temperate migratory coastal Sciaenid fish (*A. inodorus*) within the Benguela Cold Current system

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## Abstract

The Benguela Current is considered to be a major biogeographical barrier for tropical and warm-temperate marine fish, but there is limited knowledge regarding its influence on population sub-structuring of cold-tolerant species. Employing genetic variation within the mitochondrial DNA Control Region and six cross-specific nuclear microsatellite markers, a preliminary study was conducted to investigate population substructuring in *Argyrosomus inodorus*, a highly exploited, cool-temperate migratory species, across the Benguela Current region. Results revealed evidence of incipient genetic differentiation (mtDNA $\phi_{ST}$ = 0.092; nuclear $F_{ST}$ = 0.036 and $D_{ST}$ = 0.104, $P$<0.005) between the two sampling sites, suggesting the presence of two regional populations. Estimates of contemporary migration rates between populations were low, and similar in range to those reported in tagging surveys. Although preliminary, these results suggest that the oceanographic features of the Benguela Current may have influenced the evolutionary history of *A. inodorus*, and that the species is likely to be composed of two populations in the Benguela region. As the species is considered overexploited both in Namibia and South Africa, information on the distribution, population dynamics and long-term dispersal patterns across the Benguela Current region would provide a comprehensive evaluation of genetic structure, which should be incorporated into fishery management arrangements. .



**Key-words:** *Argyrosomus inodorus*, population structure, Benguela Current, isolation

## Introduction

The Benguela Cold Current system, located in the southern Atlantic, features cold sea surface temperatures bounded to the north and south by tropical currents (the Angola and Agulhas currents, respectively), and a perennial upwelling cell off central Namibia that divides the region into two sub-systems with different characteristics (Shannon 1985; Hutchings et al. 2009). The colder sea surface temperatures of the Benguela Current have been considered an important bio-geographical barrier, isolating tropical and warm-temperate fauna of the Atlantic and Indo-Pacific Oceans (Avise 2000; Floeter et al. 2008). However, recent studies revealed that other oceanographic features such as the perennial upwelling cell may also play an important role in shaping the population structure of warm-temperate fish populations within the Benguela system, as complete disruption of gene flow was documented both in *Lichia amia* and *Atractoscion aequidens* (Henriques et al. 2012; 2014). Little is known, however, regarding the influence of the Benguela system on genetic population connectivity of cold water tolerant species.

*Argyrosomus inodorus* is a migratory, benthopelagic sciaenid fish, endemic to the southeastern Atlantic (Griffiths & Heemstra 1995). Distribution range is restricted to cold-temperate waters (13ºC-16ºC), from the nearshore environment to depths of 100m, between Cape Frio and Meob Bay in Namibia, and between Cape Point and East London in South Africa (Griffiths & Heemstra 1995; Griffiths 1997; Kirchner & Holtzhausen 2001). The species distribution overlaps with those of the congeneric *A. coronus* in northern Namibia, and with *A. japonicus* along the southern and Eastern Cape coasts of South Africa(Griffiths & Heemstra 1995). Contrary to *A. inodorus*, *A. coronus* and *A. japonicus* are considered warm-temperate species, occurring preferentially in sea surface temperatures of 16º-19ºC (Potts et al 2010) and 21º-25ºC (Heemstra & Heemstra 2004), respectively. As *A. inodorus* is absent from the west coast of South Africa and there is no evidence for significant migration between the two areas of occurrence (Kirchner & Holtzhausen 2001), the species has been managed as two independent stocks. Life history characteristics appear to corroborate the hypothesis of two isolated and locally adapted populations, as features such as maximum size and size at maturity of Namibian and South African *A. inodorus* are significantly different, although no evidence of differentiation was observed within either region (Griffiths 1997; Holtzhausen et al. 2001). *A. inodorus* is a critical component of multiple coastal

fishery sectors, and exploitation pressure throughout its distribution has led to the species becoming severely depleted, with spawning stocks estimated to be 69% of unexploited values (FAO 2012). In Namibia, *A. inodorus* is harvested by the commercial and recreational fishery sectors and although approximately the same numbers of fish are captured in each sector, the average size captured in the commercial sector is larger (Kirchner 1998).While *A. inodorus* is only regulated through an input control (number of permits) the recreational fishery catch, which comprises 70% of the total recreational catch, is regulated through bag- and size-limits (Kirchner & Beyer, 1999; Holtzhausen et al. 2001). In South Africa, a 2012 survey reported that total landings of *A. inodorus* exceeded 400t per year (DAFF 2012). To establish sustainable management measures, it is necessary to understand how *A. inodorus* populations are structured across the Benguela Current region and whether migration between the two centers is absent. To date, no comprehensive genetic survey has been carried out in *A. inodorus*, with the exception of a genetic identification study in 1997 to differentiate between *A. inodorus* and *A. coronus*, based on allozymes (van der Bank & Kirchner 1997), and a more recent study on shifts of abundance of these two species in central Namibia (Potts et al. 2014).

The distribution range and life history features of this species suggest that, as observed for warm-temperate species, the oceanographic features of the Benguela Current may influence the population structure and gene flow across the region. The aim of this study was to conduct a preliminary assessment of genetic diversity, population substructuring and connectivity between the two putative populations of *A. inodorus* across the Benguela Current, using both mitochondrial DNA (mtDNA) and nuclear microsatellite DNA markers, in order to test whetherthe regional oceanographic features influence population connectivity in this cold-temperate fish species.

## Methods

### Sampling

A total of 80 fish were captured by rod-and-line fishing from the shore, by local collaborators in two areas: the West Coast Recreational Area in Namibia ($n$ = 40) and the Eastern Cape Province in South Africa ($n$ = 40), representing the two centres of

abundance of the species (Figure 1). A clip of the pectoral fin was removed immediately after capture and stored in 95% ethanol.

## Genetic screening

DNA extraction was performed using a standard phenol:chlorophorm method (Sambrook et al. 1989). Genetic variation was assessed as DNA sequence polymorphism in a fragment of the mtDNA Control Region (CR) and allele frequencies at six microsatellite loci isolated from *Argyrosomus japonicus* (Archangi et al. 2009). A total of 36 *A. inodorus* individuals were amplified by polymerase chain reaction (PCR) for CR, using primers and protocols of Appleyard et al. (2002). PCR products were purified with an enzymatic digestion, consisting of 0.5U of EXO1 (NewEngland biolabs) and 1u of shrimp alkaline phosphatase (SAP) in 1x supplied buffer (Fermentas), and sequenced in the forward direction using the same amplification primers, by Macrogen Inc. (South Korea). Sequences were visually inspected and a multiple alignment was performed in CLUSTAL X (Thompson et al. 1997), as implemented in BioEdit 7.0.1 (accession numbers: JX191998-2033).

Forty individuals per sampling site were screened at six microsatellite loci (UBA5, UBA40, UBA50, UBA91, UBA853 and UBA854). Optimized PCR mixes included 1x $NH_4Cl$ buffer, 2mM of $MgCl_2$, 0.2mM of dNTPs, 0.5pmol of each primer, 0.2U of Taq polymerase (Bioline UK) and 50-100ng of extracted DNA, in a final volume of 10 µl. The Archangi et al. (2009) protocols were modified to ensure accurate amplification: annealing temperatures and number of cycles (UBA91 $T_a$ = 52ºC, remaining loci $T_a$ = 48ºC, with 35 cycles), and removal of the final extension step of 72ºC for 10min. PCR fragments from multiple loci were combined and genotyped on an AB3500 Genetic Analyzer (Applied Biosystems). Alleles were scored as PCR product size in base pairs, and scores were determined against an internal size marker (LIZ 600), using GeneMapper 4.0 (ABIPrism). In order to ensure accurate allele size scoring between runs, individuals with known allele sizes were used in each run as positive controls.

## Data analyses

The CR dataset was assessed for levels of haplotype ($h$) and nucleotide ($\pi$) diversity, and fits to neutrality tests: Ewens-Waterson's $F$, Tajima's $D$ and Fu's $FS$, as implemented in ARLEQUIN (Excoffier et al. 2005). Determination of the most suitable nucleotide substitution model was performed in jModelTest (Posada, 2008). Preliminary inference of population connectivity of *A. inodorus* across the Benguela Current region was estimated as $\phi_{ST}$ in ARLEQUIN (Excoffier et al. 2005), with a significance level of $P < 0.05$ determined by 10,000 permutations. Haplotype networks were reconstructed to evaluate intraspecific relationships among haplotypes, using the Median-Joining (MJ) algorithm implemented in NETWORK (Bandelt et al. 1999).

Microsatellite genotypic frequencies were tested for deviation from Hardy-Weinberg expectations of random mating and from linkage equilibrium, as implemented in GENEPOP (Raymond & Rousset 1995). The occurrence of amplification errors such as large allele drop out and stuttering, and estimation of null allele frequencies were assessed in MICROCHECKER (van Oosterhout et al. 2006). Levels of genetic diversity were estimated as number of alleles (*Na*), allelic richness (*AR*), observed and expected heterozygosity ($H_O$ and $H_E$), and Wright's inbreeding coefficient ($F_{IS}$), in ARLEQUIN (Excoffier et al. 2005). A preliminary analysis to investigate the statistical power of the dataset to infer population substructuring was conducted in POWSIM (Ryman & Palm 2006). Simulations were conducted for six loci and two populations ($n$ = 40, $n$ = 40), using the estimated allelic frequencies as the baseline for the ancestral population. Runs were performed using multiple combinations of effective population size ($N_e$) and number of generations ($t$) to generate a population differentiation of $F_{ST}$ = 0.05, $F_{ST}$ = 0.02 and $F_{ST}$ = 0.01. Each simulation was run for 1,000 replicates, and power was estimated as the proportion of tests that indicated significant genetic divergence (Ryman & Palm, 2006). Genetic differentiation was measured as Weir & Cockerham (1984) $F_{ST}$ estimator, as implemented in FreeNA (Chapuis & Estoup 2007), with significance and 95% confidence intervals estimated after jackknifing. For comparison purposes, genetic differentiation was also measure using Jost's $D_{est}$ estimator, which is independent of the levels of genetic diversity, in SMOGD (Crawford 2010). Contemporary estimates of long-term average migration rates between the two sampling sites were performed for the microsatellite dataset using two complementary approaches: the classical method based on $F_{ST}$ values ($F_{ST} = 1/(4N_{em} + 1)$) (Excoffier et al. 2005), and by employing the coalescent-based approach of MIGRATE (Beerli 2009). In MIGRATE, the Bayesian approach was implemented, enforcing a full migration model, with three replicates run

for each dataset (Beerli 2009). Each analysis was performed with four connected chains, using static heating (1,000,000, 3, 1.5, 1), a burn-in period of 10,000 steps, followed by 90,000 steps, and parameters were recorded every 100 steps. Estimates of migration rates ($m$) were obtained from $M$ ($M$ = m.μ) and $\Theta$ ($\Theta = 4N_e\mu$) (Beerli, 2009). In order to obtain estimates of migration rates per generation (and not scaled by mutation) three general mutation rates were used: 0.1%, 0.5% and 1% per generation.

## Results

### Population structure and phylogeography

Sequencing of mtDNA CR yielded a fragment of 704 base pairs (bp). The 36 individuals screened displayed 32 haplotypes defined by 34 variable nucleotide sites, of which 16 sites were parsimony informative. The Tamura-Nei nucleotide substitution model was identified as the most suitable for the mtDNA dataset. Haplotype diversity was high ($h$ = 0.991), whilst nucleotide diversity was low ($\pi$ = 0.006), with Namibian samples exhibiting higher values than the South African samples (Table 1). Deviations from the assumptions of selection neutrality were observed in Fu's *FS* for both populations, but not with either Ewens-Waterson *F* or the Tajima's *D* tests (Table 1). As Fu´s *FS* is known to be sensitive to abrupt demographic changes it is likely that the observed deviation to neutrality resulted from past population size changes, rather than reflecting selection effects. Genetic differentiation ($\phi_{ST}$) between samples was low but statistically significant ($\phi_{ST}$ = 0.092, $P < 0.05$), although haplotype relationships did not show an obvious geographical pattern (Figure 2): most individuals were represented by unique haplotypes with no obvious clustering of related haplotypes into Namibian or South African groups (Figure 2). The majority of haplotypes were closely related, differing from one another by one to two mutation steps, with the exception of two HEN individuals that were divergent by 10 mutation steps (Figure 2).

None of the six microsatellite loci exhibited evidence of amplification errors, and all displayed genotype frequencies that confirmed with Hardy-Weinberg and linkage equilibrium expectations (Table 2). Levels of genetic diversity in terms of heterozygosity and allelic richness were high (overall values of $H_E$ = 0.774 and $AR$ = 13.7), with both samples displaying very similar values at individual loci and overall

(Table 2). Number of private alleles varied between 1 and 7, per locus and region (Table 2). Analyses of statistical power of the dataset revealed that the loci and sample sizes used in this study could statistically detect genetic differentiation as low as $F_{ST}$ = 0.001 in 99% of tests. As for the mtDNA data, nuclear genetic differentiation between the Namibian and South African samples was significantly greater than zero ($F_{ST}$ = 0.036, $P$<0.05), with Jost's $D_{est}$ indicating a slightly higher level of differentiation ($D_{est}$ = 0.104, $P$<0.05). Estimates of contemporary migration rates per generation between the two geographical populations were low, independently of the method used or mutation rate considered ($F_{ST}$-based: $N_{em}$ = 6; MIGRATE: $m_{2->1}$ = 0.0014; $m_{1->2}$ = 0.0011 for $\mu$ = 0.1% per generation).

## Discussion

Despite the preliminary nature of the present study, due to the limited number of sampling sites available, similarly high levels of genetic diversity and evidence for shallow but significant genetic differentiation between the two regional populations (Namibia and South Africa) of *Argyrosomus inodorus* was found. The observed mitochondrial and nuclear genetic diversity ($h$ = 0.991, $\pi$ = 0.006; $H_O$ = 0.771, $H_E$ = 0.764) were comparable with other commercially exploited fish species occurring in the Benguela Current region, such as *Argyrosomus japonicus* ($h$ = 0.96, $\pi$ = 0.009 – Klopper 2005), *Lichia amia* ($h$ = 0.991, $\pi$ = 0.006 – Henriques et al. 2012), *Atractoscion aequidens* ($h$ = 0.853, $\pi$ = 0.005; $H_E$ = 0.889 – Henriques et al. 2014) and *Rhabdosargus holubii* ($h$ = 0.91, $\pi$ = 0.006 – Oosthuizen 2007). High genetic diversity and shallow population structure are common features of marine teleosts, even in abundant, commercially exploited species. These are thought to result from historically high effective population sizes and/or high levels of gene flow between adjacent populations (Waples 1998). Interestingly, the observed genetic divergence between the Namibian and South African *A. inodorus* populations (mtDNA $\phi_{ST}$ = 0.092; nuclear $F_{ST}$ = 0.036 and $D_{est}$ = 0.104, $P$ < 0.05) was higher than that reported for other migratory sciaenids such as *Micropogonias undulatus* ($\phi_{ST}$ = 0.046 – Lankford et al. 1999) and *Sciaenops ocellatus* ($\phi_{ST}$ = 0.057 – Gold & Richardson 1998), but substantially lower than observed for other fish species with similarly disjunct distributions across the Benguela Current region (*L. amia*, $\phi_{ST}$ = 0.9 – Henriques et al. 2012; *A.aequidens*, $\phi_{ST}$ =

0.902, $F_{ST}$ = 0.055 – Henriques et al. 2014). These results, combined with estimates of the number of contemporary migrants ($N_{em}$ = 0.0014 – 6 per generation, depending on the method used), suggest a limited level of gene flow between Namibian and South African *A. inodorus* populations, and support the presence of incipient population differentiation. The present findings concur with tagging studies conducted for the species, where only two of 17,353 *A. inodorus* tagged in Namibia were recaptured in South Africa, suggesting that connectivity between populations may be limited (Kirchner & Holzhauzen 2001). Therefore, the low but significant genetic differentiation displayed by *A. inodorus* is likely to result from a present-day disjunct population distribution, with occasional migrants, and historically high levels of effective population size, rather than substantial gene flow between Namibia and South Africa.

As with other fish species distributed around southwestern Africa (e.g. *L.* amia – Henriques et al. 2012; *A. aequidens* – Henriques 2012; *Albula* spp. – Colborn et al. 2001), the distribution break in *A. inodorus* appears to correspond with the areas of cold water upwelling off southern Namibia and the west coast of South Africa (Griffiths & Heemstra, 1995; Griffiths 1997; Kirchner & Holtzhausen 2001). Although the limited sampling precludes the drawing of definitive conclusions, the reported genetic divergence and breakdown of gene flow across the Benguela Current suggests that the oceanographic features of the system, namely the cold water region, may be contributing to disrupt both adult and larval dispersal of *A. inodorus*, and supports the hypothesis of two isolated populations with limited migration between them. As the species is considered overexploited both in Namibia and South Africa, information on the distribution, population dynamics and long-term dispersal patterns across the Benguela Current region would provide a comprehensive evaluation of genetic structure, which should be incorporated into fishery management arrangements.

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## Tables

**Table 1:** Estimates of mitochondrial genetic diversity levels and neutrality tests for *A. inodorus* CR: *n* – number of individuals; *H* – number of haplotypes; *h* – haplotype diversity; $\pi$ - nucleotide diversity; *F* – Ewens-Waterson neutrality test; *D* – Tajima neutrality test; *FS* – Fu neutrality test. Significant departures from expectations ($P < 0.05$) in bold.

| | **HEN** | **EastC** | **Overall** |
|---|---|---|---|
| ***n*** | 18 | 18 | 36 |
| ***H*** | 18 | 14 | 32 |
| ***h*** | 1.000 | 0.968 | 0.991 |
| $\boldsymbol{\pi}$ | 0.008 | 0.004 | 0.006 |
| ***F*** | - | 0.862 | 0.966 |
| ***D*** | -1.486 | 0.324 | -1.554 |
| ***Fs*** | **-14.762** | **-10.099** | **-25.652** |

386

387 **Table 2:** Genetic diversity in *A. inodorus* samples at six cross-specific microsatellite
388 loci: *n* – number of individuals genotyped; *NA* – number of alleles; *AR* – allelic
389 richness; *PA* – number of private alleles $H_E$ – expected heterozygosity; $H_O$ – observed
390 heterozygosity; $F_{IS}$ – inbreeding coefficient. Significant deviations to Hardy-Weinberg
391 expectations in bold.

| | | HEN | EastC | Overall |
|---|---|---|---|---|
| | ***n*** | 40 | 40 | 80 |
| | ***NA*** | 11 | 11 | 13 |
| | ***AR*** | 10.803 | 10.925 | 10.52 |
| **UBA5** | ***PA*** | 1 | 2 | 3 |
| | $\boldsymbol{H_E}$ | 0.819 | 0.825 | 0.839 |
| | $\boldsymbol{H_O}$ | 0.875 | 0.825 | 0.850 |
| | $\boldsymbol{F_{IS}}$ | -0.047 | 0.003 | -0.007 |
| | ***n*** | 40 | 39 | 79 |
| | ***NA*** | 8 | 7 | 8 |
| | ***AR*** | 7.951 | 7.000 | 7.452 |
| **UBA40** | ***PA*** | 1 | 0 | 1 |
| | $\boldsymbol{H_E}$ | 0.765 | 0.807 | 0.790 |
| | $\boldsymbol{H_O}$ | 0.750 | 0.795 | 0.772 |
| | $\boldsymbol{F_{IS}}$ | 0.038 | 0.037 | 0.028 |
| | ***N*** | 39 | 40 | 79 |
| | ***NA*** | 14 | 15 | 16 |
| | ***AR*** | 13.974 | 14.899 | 14.846 |
| **UBA50** | ***PA*** | 1 | 2 | 3 |
| | $\boldsymbol{H_E}$ | 0.887 | 0.896 | 0.914 |
| | $\boldsymbol{H_O}$ | 0.821 | 0.800 | 0.810 |
| | $\boldsymbol{F_{IS}}$ | 0.066 | 0.038 | 0.120 |

| | | | | |
|---|---|---|---|---|
| | ***n*** | 40 | 40 | 80 |
| | ***NA*** | 5 | 3 | 5 |
| | ***AR*** | 4.902 | 3.000 | 3.962 |
| **UBA91** | ***PA*** | 1 | 1 | 2 |
| | $H_E$ | 0.361 | 0.387 | 0.375 |
| | $H_O$ | 0.275 | 0.475 | 0.375 |
| | $F_{IS}$ | 0.182 | -0.207 | 0.006 |
| | ***n*** | 40 | 40 | 80 |
| | ***NA*** | 13 | 14 | 17 |
| | ***AR*** | 12.799 | 12.924 | 14.530 |
| **UBA853** | ***PA*** | 3 | 4 | 7 |
| | $H_E$ | 0.831 | 0.872 | 0.876 |
| | $H_O$ | 0.925 | 0.900 | 0.913 |
| | $F_{IS}$ | -0.100 | -0.036 | -0.035 |
| | ***n*** | 40 | 40 | 80 |
| | ***NA*** | 9 | 7 | 19 |
| | ***AR*** | 7.604 | 11.899 | 15.152 |
| **UBA854** | ***PA*** | 7 | 1 | 8 |
| | $H_E$ | 0.881 | 0.776 | 0.860 |
| | $H_O$ | 0.975 | 0.675 | 0.825 |
| | $F_{IS}$ | -0.038 | 0.130 | 0.047 |
| | ***n*** | 40 | 40 | 80 |
| | ***NA*** | 10 | 9.500 | 11.333 |
| | ***AR*** | 9.004 | 9.833 | 13.667 |
| **Average all loci** | ***PA*** | 14 | 10 | 24 |
| | $H_E$ | 0.757 | 0.760 | 0.776 |
| | $H_O$ | 0.770 | 0.745 | 0.758 |
| | $F_{IS}$ | 0.000 | 0.012 | 0.027 |

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## Figure Legends

**Figure 1:** Sampling strategy for *A. inodorus* across the Benguela Current region, highlighting sampling sites, and their position relative to the major oceanographic features of the system: position of the Benguela and Agulhas Currents, central Namibia upwelling cell, and continental platform width.

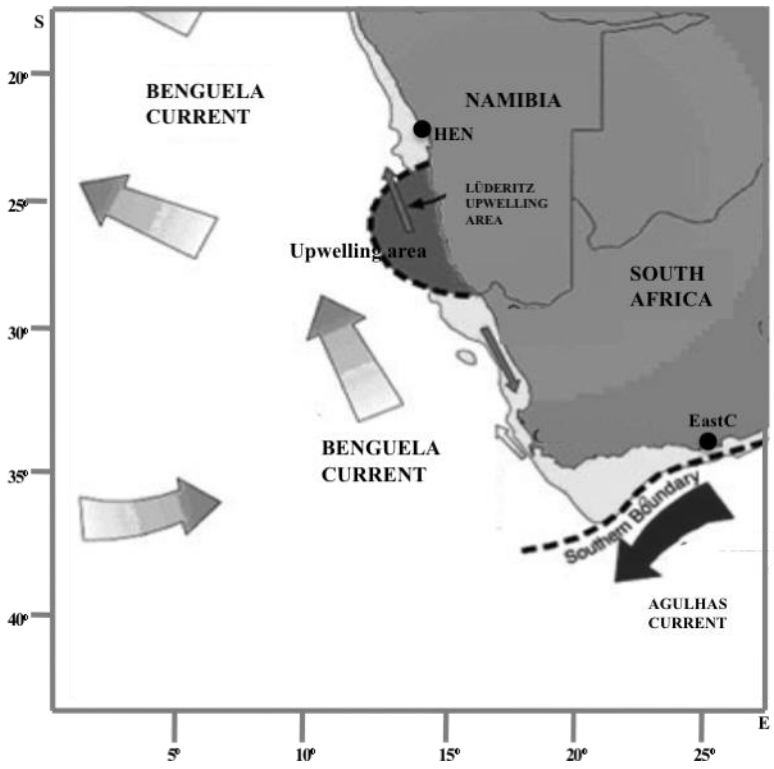

**Figure 2:** Haplotype network for *A. inodorus* across the Benguela Current region, based on 704bp of mtDNA CR sequences: ⊖ = HEN; ◍ = EastC. Branch lengths are proportional to number of nucleotide differences, and node sizes are proportional to the number of individuals. Red dots represent unsampled inferred haplotypes.